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Journal of Chromatography A, 1056 (2004) 121–130
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Strategic sample composition in the screening of polycyclic aromatic hydrocarbons in drinking water samples using liquid chromatography with fluorimetric detection

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Available online 19 June 2004

Abstract

The screening of polycyclic aromatic hydrocarbons in water samples by means of the strategic sample composition (SSC) technique is presented. SSC uses especial supersaturated composition matrices to perform sample composition and analysed the results obtained in the analytical determination of these composite samples by means of evolutionary assisted regression procedures providing estimations of the concentration levels of analytes in each individual sample specimen. Here, 12 composite samples were prepared by departing from 26 water sample specimens, five of which were spiked with known amounts of several polycyclic aromatic hydrocarbons (PAHs). These composite samples were analysed by HPLC using fluorescence detection. Concentration levels spiked were, in some cases clearly higher than allowed limits for drinking waters, whereas in other cases are just in the limit or even down these limits. The study shows the ability of SSC to detect the really contaminated samples and guiding the analyst in taking the adequate decisions.

Keywords: Water analysis; Sample composition; Polynuclear aromatic hydrocarbons

1. Introduction

Widespread in the environment, polycyclic aromatic hydrocarbons (PAHs) are priority pollutants on both UE [1] and EPA [2] lists. PAHs enter the environment mostly as releases to air from volcanoes, forest fires, residential wood burning, and exhausts from automobiles and trucks. They can also enter surface water through discharges from industrial plants and wastewater treatment plants, or released to soils at hazardous waste sites due to escapes from storage containers. In the marine compartment, petroleum inputs are significant as a consequence of river discharges, accidental crude oil spills, ballast operations, sewage disposal, offshore production and transport. PAHs are of great concern since some components are mutagenic or carcinogenic [3], so selective and sensitive analytical procedures are required to control these pollutants at very low level in drinking, surface and waste water. Systematic monitoring is applied in some countries and, frequently, unexpected pollution episodes impose extensive sampling campaigns and intensive monitoring to evaluate the magnitude of suspected environmental impacts. Thus, PAHs screening, as with many other persistent pollutants, is characterised by large sample inputs that requires the application of lengthy and costly sophisticated sample preparation schemes, followed by highly sensitive analytical measurements (usually GC–MS or HPLC–FD).

A conventional approach to alleviate the analytical workload in these screening campaigns is sample composition [4,5]. Sample composition is useful when the cost of testing is higher than the cost of sampling. Conventional sample composition reduces the cost of testing by physically mixing individual sample specimens to form the composite samples. Then composites are tested instead of the individual sample specimens. If the goals of the screening are only the estimation of average population representative values, the results for composite samples provide the required information. However, in most cases, also the information about contaminated individual sample specimens (hot spots) is needed so results for composite samples mean only a way to detect the presence or absence of contaminated sample

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0021-9673/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.chroma.2004.04.066

specimens in the burden of the sampling campaign inputs. Whenever a composite sample appears positive, all the individual sample specimens entering this composite sample must be individually tested to see how many were really contaminated and to estimate their pollution levels.

In real life cases, if particularised information on sample specimens is needed, composite sampling is only advantageous if we have reasons to assume that only some of the original sample specimens are really above the concentration limits established for the target analytes. This is a way to express the named Pareto principle or the effect sparsity. This means that in environmental screening studies we are searching for hot spots or outliers in the large number of sample specimens representing the area under study, and the average value for the area lacks interest. In these circumstances, conventional sample composition may provide little or no advantages over the individual analysis approach.

Recently, a new approach named strategic sample composition [6–13] (SSC) has been developed to reduce drastically the analytical effort while simultaneously providing individualised information on sample specimens, thus avoiding in most cases the need of re-analysing. SSC makes an extensive use of supersaturated design matrices [14–16] to perform sample composition and to evaluate the concentration level of the analytes in the original sample specimens through the analysis of the composite samples formed. SSC is especially advantageous if slow, labour-intensive pre-processing, separation and measurement processes need to be applied. Many chromatographic procedures applied systematically in environmental and quality control screening can benefit from the reduction in costs and time provided by SSC. SSC can be applied both in manual and automatic modes, although fully automation allows SSC to provide the best performance.

An important aspect in SSC is the robustness of the procedure. This feature derives from the structure of the design matrix applied to form the composite samples and has two important consequences. First, no replicate measurements are usually necessary for composite samples. This factor helps to reduce the analytical workload, increasing the sample throughput. Second, SSC can accept accidental errors without significantly loosing its prediction ability.

In this paper, the features of SSC as applied to the control of polycyclic aromatic hydrocarbons in drinking water samples are presented. A specially developed device whose main features are also presented provides full automation of the sample composition process.

2. Experimental

2.1. Reagents and materials

Acetonitrile (gradient-grade), dichloromethane (pesticide-grade), and 2-propanol (analysis-grade) were obtained from Merck (Darmstadt, Germany). Standards of aromatic polycyclic hydrocarbons: naphthalene, acenaphthene, fluorene, phenantrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo (k)fluoranthene, benzo(a)pyrene, Benzo(ghi)perylene and Indeno(1,2,3-c,d)pyrene, were purchased from Supelco (Bellafonte, PA, USA), and they were of 99% purity. Individual standards were prepared in acetonitrile/dichloromethane (1:1). Standards for calibrations were prepared by first diluting concentrated stocks in acetonitrile and finally, diluting in acetonitrile/water (1:1) to appropriated concentration levels. Anhydrous sodium sulphate was purchased from Scharlau (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q system purchased from Millipore (Bedford, MA, USA). Cellulose ester filters (SMWP, 47 mm, 5 μm) and Durapore filters (Millex GV, 13 mm, 0.22 μm) were supplied from Millipore.

2.2. Apparatus

The high-performance liquid chromatography system consisted of a 600E pump (Waters, Milford, MA, USA), a diode array detector (DAD) and a fluorescence detector HP Series 1100 (Agilent, Waldbronn, Germany) connected in series. The injector was a Rheodyne, Model 7725i (Cotati, CA, USA), fitted with a 20 μL external loop. The temperature of the column was controlled by means of a MetaTherm 9540 oven, supplied by MetaChem (Torrance, CA, USA).

Chromatographic separation was performed with a 250 mm × 2.1 mm i.d. Vydac 201 TP52 column, with a particle size of 5 μm, supplied by Agilent. A 30 mm × 2 mm guard column Vydac 201 TP was employed to protect the analytical column.

Data acquisition was carried out by means of HP Chemstation Software (Agilent, Rev. A. 06.03 [509]). Sample extracts were concentrated using a TurboVap II nitrogen evaporator supplied by Zymark (Hopkinton, MA, USA).

Sample composition was performed in the device shown in Fig. 1. It is constructed using two identical turntable samplers adapted from commercial automatic titration devices (AS 20 samplers, supplied by Crison Instruments, Barcelona, Spain). Only arms adaptors needed small modifications to hold the extremes of the pipes to perform sample composition. Sampler in the left (Sampler 1) is used to hold the original sample specimens while the sampler at the right (Sampler 2) is for composite samples. A standard automatic dispenser (Crison) fitted with a 10 mL Hamilton (Hamilton Bonaduz, AG, Switzerland) syringe completes the device. The system uses glass tubes of 40 mm × 200 mm (mod V60123, Afora, Barcelona, Spain) to accommodate the sample specimens and the composite samples. Position 20 in both samplers is reserved for special functions. In sampler 1, tube 20 is filled with clean solvent and used to rinse the tip of the sampler and the pipelines when a new sample specimen is to be processed. In sampler 2, tube 20 is bottom holed and used to drain waste liquids to a high volume waste bottle container, through a special recipient (mod V60126, Afora, Barcelona, Spain) as shown in the scheme in the right upper

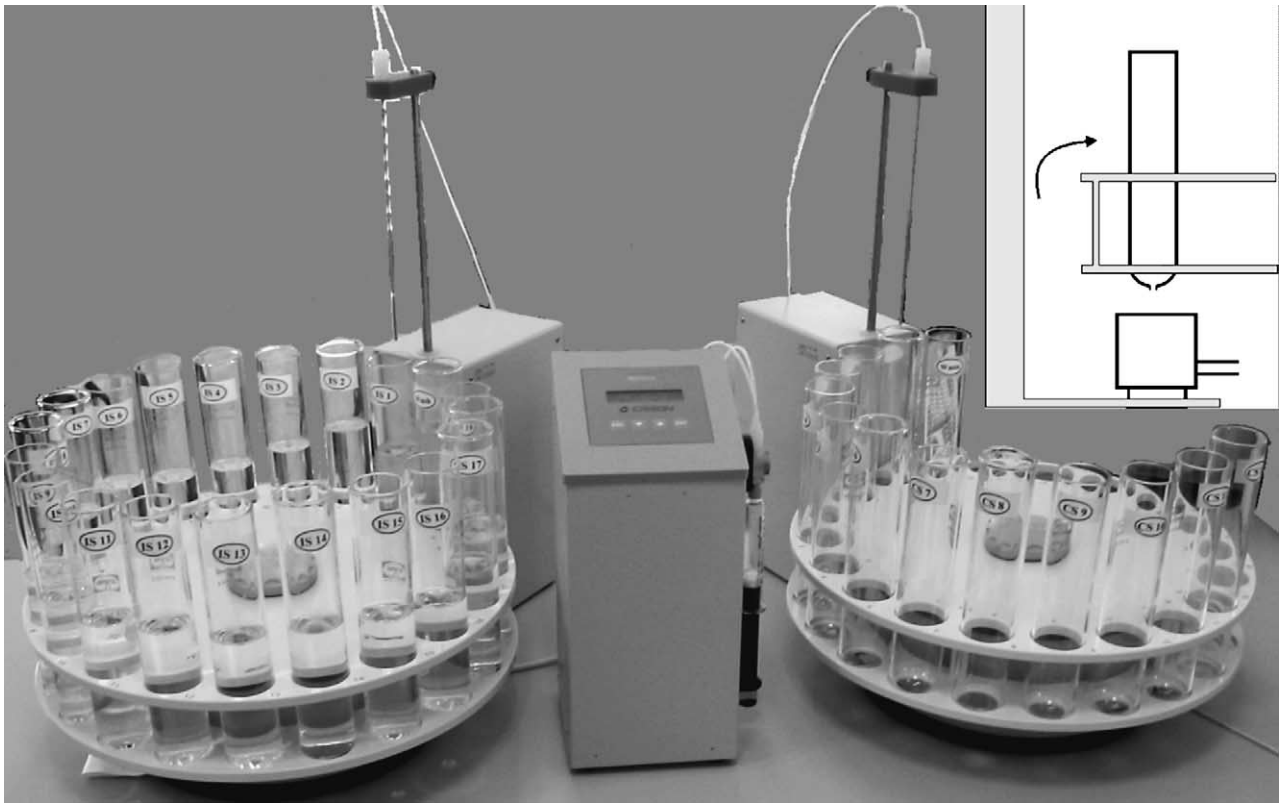


Fig. 1. View of the device used for the automatic sample composition of liquid samples following SSC principles. Upper right part shows a scheme of the drainage tube in sampler 2.

part of Fig. 1. The whole system is controlled by a software application specially developed with this aim.

2.3. Handling of individual sample specimens and sample composition

To avoid losses of PAHs during storage and manipulation and ensure consistent recoveries, 2-propanol was added to an organic solvent miscible with water must be added to the sample, specimens in a proportion of 10% [17]. Also clean up solvent used to rinse the system between samples changeover contains a 10% of 2-propanol to avoid sample carryover.

A total number of 26 sample specimens were prepared by measuring 1 L of filtered drinking water through a 5 μm cellulose ester filter and adding 100 mL of 2-propanol. Five of these sample specimens were randomly selected and spiked with variable amounts of up to five PAHs. Spiking levels were adjusted to obtain concentration levels in the samples bellow, above and close to the limits allowed for drinking water according to European regulations. Not all spiked sample specimens received the same pollutants to mimic sample specimens belonging to a real sampling campaign where it is expected significant differences between samples.

Sample composition was carried out using the composition matrix showed in Table 1. Equal volume aliquots of 6 mL of each sample were added to the corresponding composite sample, except for sample 8 (S_8) and sample 16 (S_{16}), from which 12 mL were taken to check proportional sample composition capabilities of the technique. Final volumes of composite samples were not made constant. The SSC software accounts for the final volumes in each composite sample in order to compensate volume differences at the calculations stage. Once the fully automatic composition process has finished, composite samples were stored in 250 mL amber glass bottles at 4 °C until they were analysed.

2.4. Sample extraction

The whole volume of prepared composite samples were submitted to liquid-liquid extraction. In order to have comparative results, 250 mL of original sample specimens were also extracted and analysed.

Each sample was transferred to a 1 L separation funnel and an aliquot of dichloromethane was added (20 mL for composite samples and 30 mL for the original sample specimens). This aliquot of dichloromethane was previously used to wash the inner surfaces of the bottle in which the sample was stored. The separation funnel was vigorously shaken for 2 min. The organic layer was allowed to separate from water phase, and collected in a 250 mL Erlenmeyer flask. The extraction procedure was repeated two more times with identical extractant aliquots. After drying the extract with

Table 1
Supersaturated matrix used in the sample composition of water samples for PAHs screening

	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	S_{16}	S_{17}	S_{18}	S_{19}	S_{20}	S_{21}	S_{22}	S_{23}	S_{24}	S_{25}	S_{26}
CS_1	1	0	0	1	0	1	0	0	1	1	0	0	0	1	0	1	0	1	0	1	0	0	1	1	1	1
CS_2	0	0	0	0	0	1	1	0	0	1	1	1	1	0	0	1	1	0	1	0	0	0	1	0	0	1
CS_3	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	1	1	1	1	0	1	1	0	1	0
CS_4	0	0	1	0	0	1	0	1	1	0	1	0	1	1	0	0	1	1	0	0	1	1	0	1	0	1
CS_5	0	0	0	1	1	0	1	0	0	1	0	0	1	1	1	0	0	0	1	0	1	0	0	1	1	1
CS_6	0	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	1	0	1	1	1	0	1	1
CS_7	0	1	1	0	0	0	1	0	0	1	1	1	0	0	1	0	0	1	0	1	0	1	0	1	1	0
CS_8	1	0	0	0	1	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	1	0	0	1	0	0
CS_9	1	1	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1	0	0	0
CS_{10}	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
CS_{11}	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CS_{12}	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

anhydrous sodium sulphate, it was concentrated to 0.5 mL under nitrogen stream. Then the residual solvent was exchanged to acetonitrile, and the extract was evaporated again to 0.5 mL. Finally, the extract was transferred to a 2 mL volumetric flask, 0.5 ml of acetonitrile added, and the flask volume was made to the mark with ultrapure water. The extract was filtered through a 0.22 μm Durapore filter (Millipore Co., Bedford, MA, USA) and 20 μL were injected in the HPLC coupled with fluorescence and photodiode array detectors.

2.5. Chromatographic conditions

Acetonitrile and water were used as eluents at a flow-rate of 0.4 mL min^{-1}. The gradient elution program profile has been summarised in the caption to Fig. 2 that shows a typical chromatogram of the standard's mixture as registered in the optimised experimental conditions. The column temperature was maintained at 35 °C.

Fluorescence detector was programmed in order to get a good sensibility for all the peaks. Thus, eight segments of excitation/emission wavelength pairs were programmed as summarised in Table 2. The diode array spectrophotometer was eventually used to confirm the identity of the peaks, by comparing the obtained spectra with those referenced in the spectral library. It was used over a wavelength range 190–400 nm.

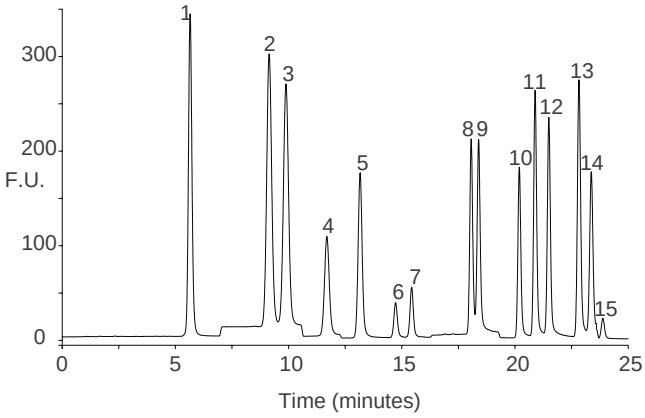


Fig. 2. Chromatogram of a standard mixture of PAHs as produced in the elution and detection conditions given in Table 3. Compounds: (1) naphthalene; (2) acenaphthene, (3) fluorene; (4) phenantrene; (5) anthracene; (6) fluoranthene; (7) pyrene; (8) benzo(a)anthracene; (9) chrysene, (10) benzo(b)fluoranthene; (11) benzo(k)fluoranthene; (12) benzo(a)pyrene; (13) dibenzo(a,h)anthracene; (14) benzo(g,h,i)perylene; (15) indeno(1,2,3-c,d)pyrene. Elution program: multisegmented in 45 min, using linear transitions from steps starting at 47% (2.0 min), to 90% in 10 min and to 100% of acetonitrile in 2 min).

2.6. Data analysis

The design matrix used in this work to prepare the composite samples was obtained by means of a dedicated program package, named Superga® [7], which allows one to

Table 2
Fluorescence program for the detection of PAHs in the conditions of gradient corresponding to Fig. 2

Time (min)	Excitation wavelength (nm)	Emission wavelength (nm)	Compounds detected in program segment
0.0	267	330	Naphthalene
7.0	275	315	Acenaphthene, fluorene
10.6	247	357	Phenantrene
12.3	238	418	Anthracene, fluoranthene, pyrene
16.3	267	385	Benzo(a)anthracene, chrysene
19.3	260	420	Benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene
22.4	285	404	Dibenzo(a,h)anthracene, benzo(g,h,i)perylene
23.65	245	500	Indeno(1,2,3-c,d)pyrene

build optimal supersaturated matrices specially adapted for sample composition purposes.

Sample composition and evaluation was performed by the dedicated application Gamich [7]. In its current version, this program package is composed by three applications covering all the aspects of strategic sample composition. Module SSC Manager® is used to develop the sample composition process and to manage the automatic device as well as to guide all the steps through the process. SSC Solver® allows the evaluation of the concentrations of analytes in the original sample specimens applying a regression mechanism driven by evolutionary algorithms [7]. Supersat® is the third element in Gamich used for the general resolution of supersaturated matrices. These software packages were developed and implemented in the laboratory using CA-Realizer 3.0A (Computer Associates) for Superga® and Delphi 7 (Borland Co) language for Gamich.

3. Results and discussion

3.1. Basic steps in the sample composition process

Strategic sample composition (SSC) is a technique developed to reduce the work and cost spent in screening studies where the Pareto principle (or effect sparsity) is likely to occur. The fundamentals of SSC have been detailed elsewhere [8–12], so here only a brief description of the process will be made. In SSC composite samples are prepared by following the directives of supersaturated matrices instead of by simple mixing of the original sample specimens. SSC composition matrices (e.g. the one in Table 1) have as many rows as experiments (the number of composite samples to prepare, Cs_1, ..., Cs_N), and as many columns as original sample specimens (S_1, ..., S_M). The matrix uses the common '0' and '1' coding, meaning that a particular sample specimen (S_i) will be present (state 1) or absent (state 0) in any particular composite sample (Cs_j). As can be seen in Table 1, the last row in the composition matrix have all sample specimens in the 1 state, meaning that this composite sample will be formed by all the original sample specimens thus resembling a conventional composite sample. Another particularity of this composite sample is that represents the maximum dilution in the composition process because all the specimens enter in the mixture. The remaining composite samples are less diluted (approximately by a factor of 2 as compared to the last one), and should be analysed directly although the final volumes can also be made equal.

As a consequence, in SSC technique the Cs_N composite sample should be analysed firstly. If the analysis of this composite sample produced negative (background) results we will conclude that no original sample specimen was polluted and the remaining composite samples can be discarded. In these circumstances, SSC retain all the advantages of conventional sample composition. On the contrary, if the Cs_N analysis produced a positive result, the remaining composite samples (Cs_1 to Cs_{N-1}) need to be analysed but not the original sample specimens. In this way we know beforehand the maximum number of analyses that have to be carried out for a set of sample specimens.

Of course, the conclusions above mentioned are based on the confidence that the probability of false negatives in the analysis of the Cs_N composite sample is sufficiently low. In order to warrant this probability level, care must be taken to avoid excessive dilution. Moreover, it is advisable to analyse this Cs_N sample at least by duplicate.

Consequently, the maximum allowable dilution determines the number of specimens in the composition matrix and it is used to decide which composition matrix will be selected. A second factor in the selection of the composition matrix is the correlation between matrix columns. Because we use supersaturated matrices, correlation be-

Table 3
Figures of merit for the analytical procedure applied in the screening of PAHs in water samples

Compound	Quantification limit in samples (ng/L) (signal to noise ratio 10)	Correlation coefficient in the calibration line	Reproducibility. Coefficient of variation ($n = 6$ in different days)		Recovery (%) ($n = 6$)
			Retention times	Area	
Naphthalene	4.3	0.9990	0.9	4.1	53 ± 10
Acenaphthene	4.4	0.9990	1.2	3.8	54 ± 9
Fluorene	0.5	0.9996	1.3	0.5	51 ± 10
Phenantrene	0.2	0.9999	1.2	3.0	52 ± 10
Anthracene	0.2	0.9999	0.9	2.9	62 ± 9
Fluoranthene	0.3	0.9999	0.7	2.0	78 ± 7
Pyrene	0.1	0.9994	0.6	7.0	81 ± 9
Benzo(a)anthracene	0.5	0.9998	0.4	3.5	97 ± 5
Chrysene	0.6	0.9998	0.3	1.7	95 ± 5
Benzo(b)fluoranthene	0.4	0.9999	0.2	3.8	100 ± 4
Benzo(k)fluoranthene	0.2	0.9999	0.2	3.4	101 ± 4
Benzo(a)pyrene	0.1	0.9999	0.2	10.5	103 ± 5
Dibenzo(a,h)anthracene	0.3	0.9976	0.2	4.0	104 ± 4
Benzo(g,h,i)perylene	0.4	0.9995	0.2	9.3	102 ± 4
Indeno(1,2,3-c,d)pyrene	1.0	0.9995	0.5	4.0	104 ± 4

tween columns is unavoidable. Supersaturated matrices are constructed [13,14] to be '*as orthogonal as possible*' but correlation grows on increasing the ratio of columns to rows in the matrix. The experience with SSC has shown that composition matrices having column-to-row ratios greater than 2 are not advisable for practical purposes.

Table 3 shows the figures of merit of the analytical procedure used to analyse the composite samples. The worst value of the limits of quantification can be used to define the maximum allowable dilution and thus, the maximum number of sample specimens in the sample composition process. The objective is to warrant that if only one sample specimen in the set is polluted clearly above this limit, SSC would be able to detect the presence of this polluted sample in the mixtures. If we consider all the compounds listed in Table 3, this limit would be imposed by acenaphthene. The European Union has adopted limit values from drinking water regarding only to five of these compounds [1]. These limits are 0.01 ng/mL for benzo(a)pyrene and 0.1 ng/mL for the sum of concentrations of benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene and indeno(123-cd)pyrene. Thus, considering only the PAHs and the defined limits in European regulations the maximum allowable dilution in our case would be imposed by indeno/1,2,3-c,d)pyrene and benzo(a)pyrene. Because the dilution factor appear no critical, we opted by a design matrix that slightly surpasses the column-to-row 2:1 limits, and decided to form the composite samples using a set of 26 sample specimens. In this way the probability of false negatives in the analyses of the Cs_N is maintained at a reasonable low level while the factor of analytical trials reduction is maintained over two.

3.2. Linear or proportional composite samples

In many cases in conventional sample composition, all sample specimens are mixed in equal proportions but sample composition involving variable proportion of discrete sample specimens is important in many environmental and geochemical studies [15]. SSC was designed to handle linearly mixed composite samples as well as proportional composite samples. In order to define a proportional composition scheme we only need to establish a unit volume of sample and then associate to each sample specimen a proportion coefficient. In our study two sample specimens were selected to enter the composite samples in twice as much proportion. These sample specimens were S_8 and S_{16}. This assignment was made to mimic a real case were some suspected more diluted samples are entered in higher proportion in the composites.

3.3. Automation of the sample composition process

Although SSC can be applied without having any special software or automation tools, the automation of the sample composition process is of utmost importance to avoid the risk of human mistakes carrying out a composition matrix that involves handling a large number of sample specimens and composite samples. Automation warrants the absence of composition errors freeing at the same time the analyst from that tedious task.

The device shown in Fig. 1 has been specifically designed to drive strategic sample composition in a fully automatic mode. The module SSC Manager® controls the device so the user need only to define the options related to the clean up cycles of system on sample changeover. The system mixes the amounts of the original sample specimens defined by the base volume and the corresponding proportion coefficient.

Actual device in Fig. 1 allows the easy handling of any number of sample specimens in multiples of 19 in sampler 1 (position 20 in sampler 1 is reserved for clean up service), and a maximum of 19 composite samples in sampler 2 (also position 20 in sampler 2 is reserved for draining purposes). It is not very usual to prepare more than 19 composite samples but very often we have more than 19 sample specimens. Thus, the system must be refilled with new tubes containing

Table 4
Results in the analytical determination of composite samples prepared according the composition matrix in Table 1

Composite sample	Concentration (ng/mL)				
	Benzo(b)fluoranthene	Benzo(k)fluoranthene	Benzo(a)pyrene	Benzo(g,h,i)perylene	Indeno(1,2,3-c,d)pyrene
Cs_1	0.029	0.044	0.027	0.049	0.027
Cs_2	0.013	0.006	0.003	0.010	0.006
Cs_3	0.047	0.057	0.025	0.040	0.045
Cs_4	0.010	0.024	0.005	0.016	0.023
Cs_5	0.052	0.057	0.034	0.050	0.036
Cs_6	0.022	0.023	0.003	0.022	0.030
Cs_7	0.007	0.025	0.004	0.013	0.028
Cs_8	0.015	0.009	0.009	0.026	0.009
Cs_9	0.011	0.036	0.008	0.013	0.029
Cs_{10}	0.042	0.045	0.026	0.036	0.025
Cs_{11}	0.035	0.045	0.029	0.036	0.022
Cs_{12}	0.025	0.034	0.014	0.024	0.027
Cs_{12} (replicate)	0.022	0.030	0.012	0.020	0.023

blocks of 19 sample specimens when processing of the previous ones has finished. Another important limitation in this device is the total volume of the tubes that can be handled, which currently is 200 mL. This limits, in turn, the maximum volumes of sample specimen aliquots that can enter the composite samples so highly sensitive analytical procedures are needed to assure detection capability in composite samples. All these factors must be considered before starting a new sample composition project.

3.4. Screening polycyclic aromatic hydrocarbons in water samples by SSC

Table 4 summarises the results in the analyses of the composite samples formed to screen PAHs in the set of 26 sample specimens considered. From these data, SSC solver performs the regression on the composition matrix and predicts the concentrations of analytes in each original sample specimen as well as the sum of PAHs in the samples.

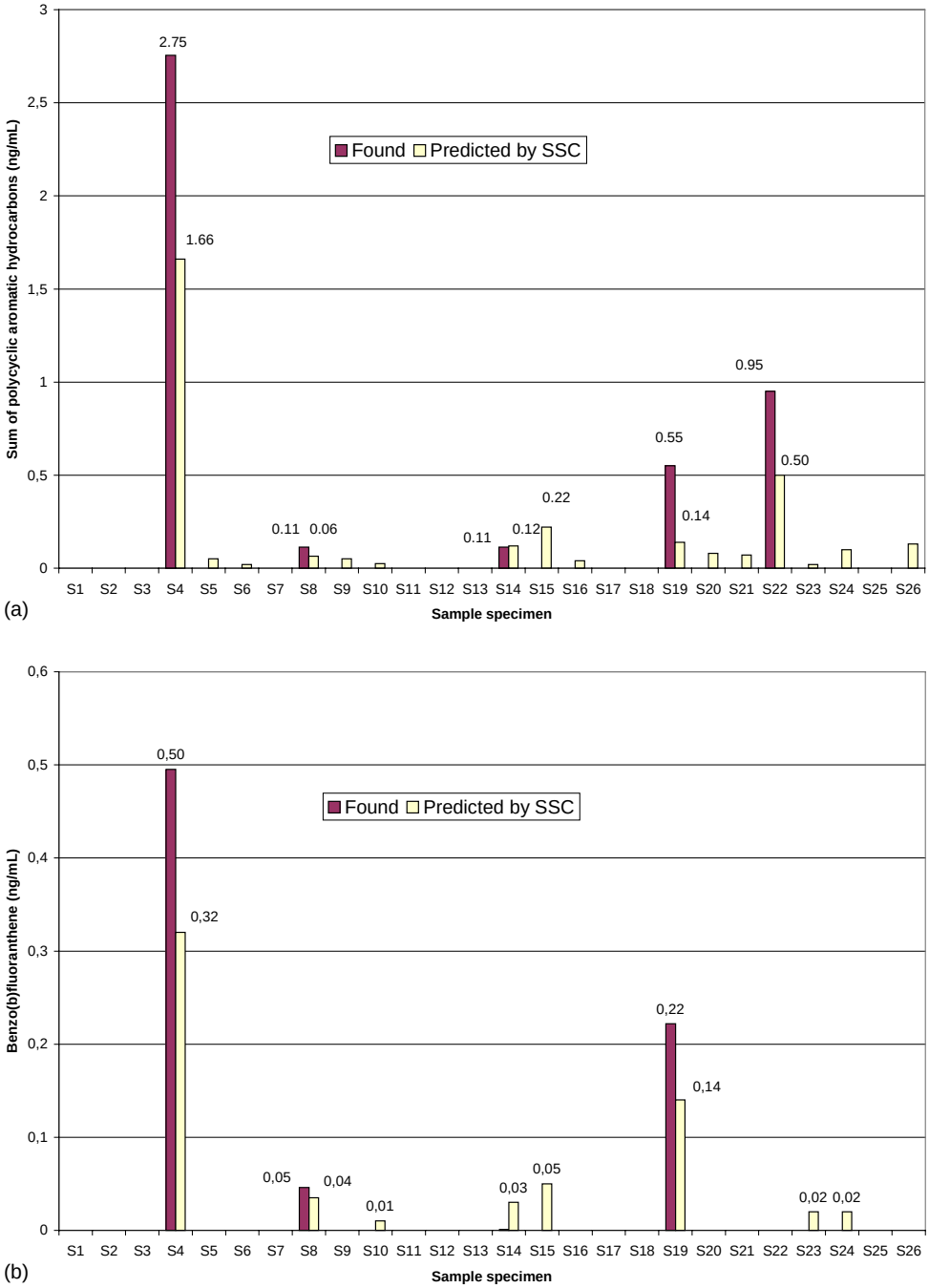
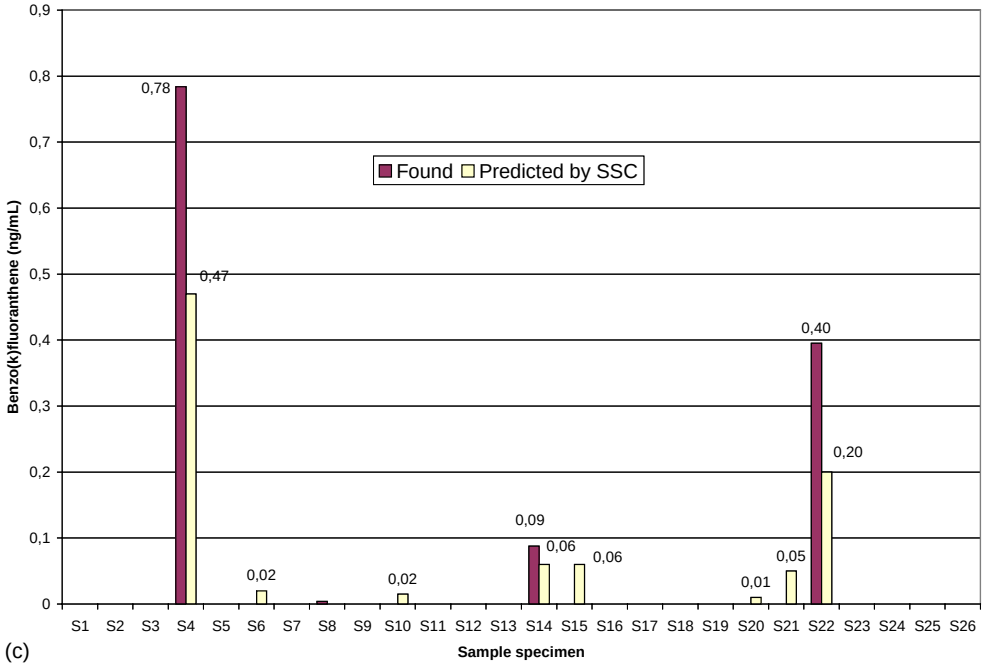


Fig. 3. Comparison between predicted concentration levels for the PAHs considered and found values on analysing the spiked sample specimens: (a) Sum of PAHs; (b) results for benzo(b)fluoranthene; (c) results for benzo(k)fluoranthene; (d) results for benzo(a)pyrene; (e) results for benzo(g,h,i,)perylene; and (f) results for indeno(1,2,3-c,d)pyrene.



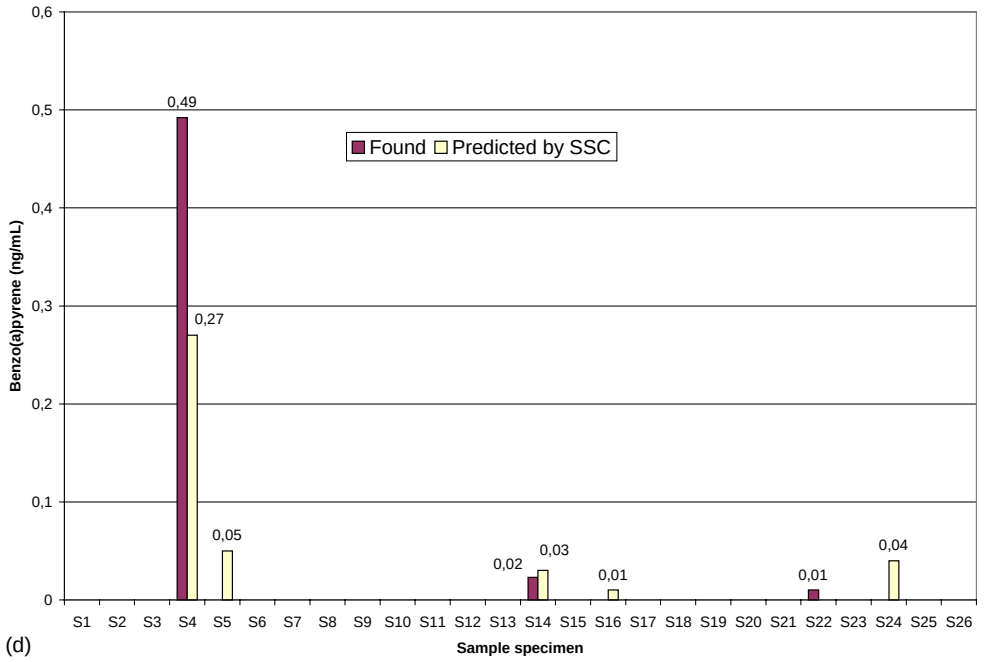
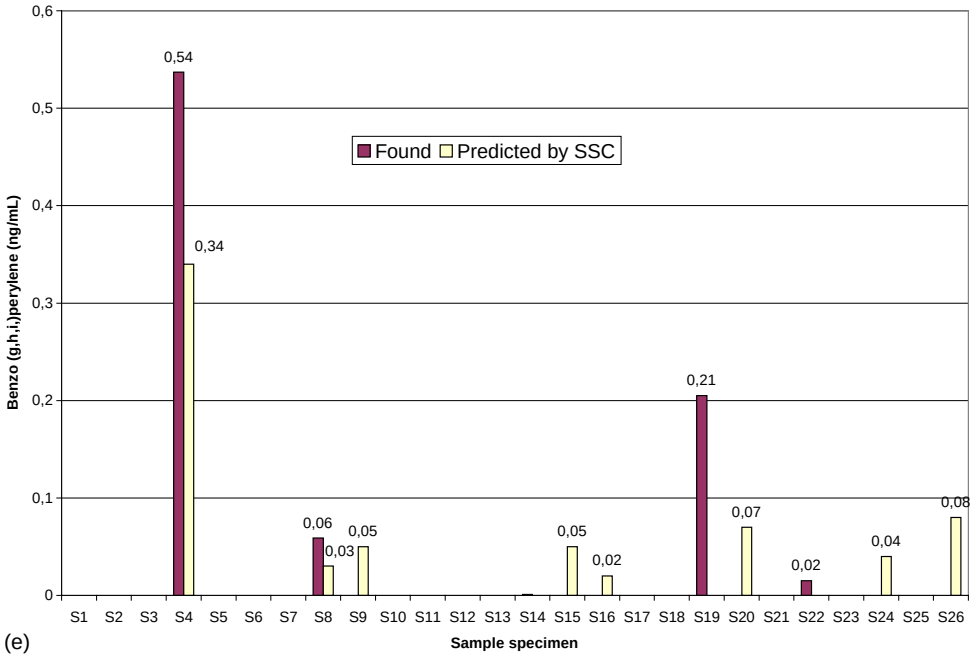


Fig. 3. (*Continued*).

Because the results for Cs_{12} composite sample clearly indicated the presence of polycyclic aromatic hydrocarbons in the sample specimens, the remaining composite samples were analysed. Composite sample Cs_{12} was prepared and analysed in duplicate to check consistency of analytical results and to limit the risk of false negatives.

These results have been organised in the graphs of Fig. 3. Fig. 3a shows the comparison of predicted sums of PAHs and the experimental values obtained in the analysis of sample specimens (it should be noticed that only spiked sample specimens were analysed individually). In the set of 26 sample specimens three samples (S_4, S_{19} and S_{22}) clearly outscores the regulated level of 0.1 ng/mL. In general, due to non-negativity constraints imposed in the regression technique [7] applied to solve the composition matrices in SSC, predictions are generally under real concentration values so the probability of false negatives for samples just in the limit is significant. However, SSC predicts not only the sum of analytes but the concentrations of each one in the sample specimens, so additional information can be obtained by



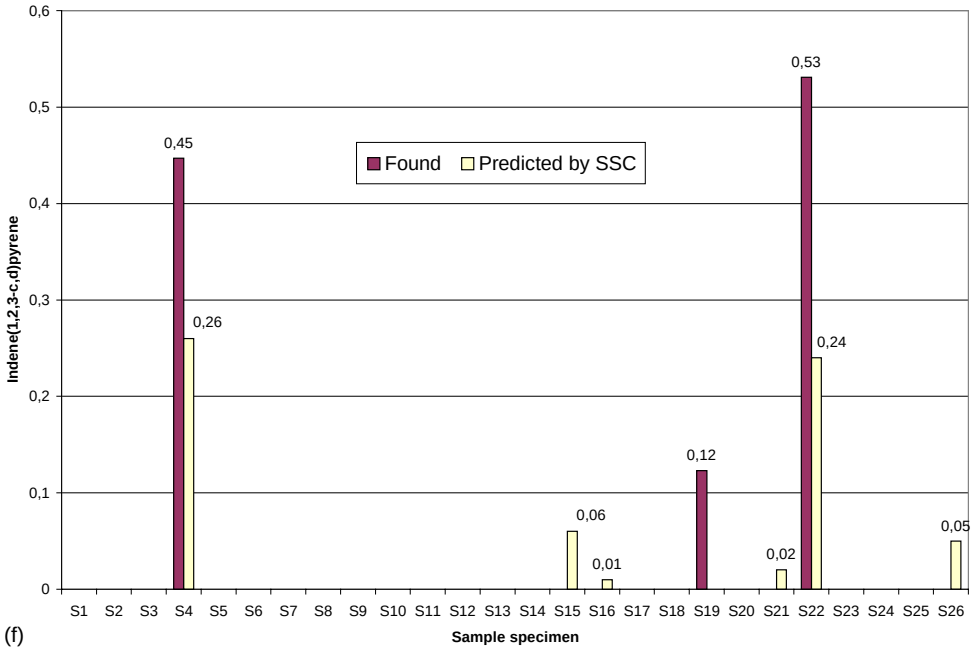


Fig. 3. (*Continued*).

analysing the results for the individual analytes. These results are shown in the graphs of Fig. 3b-f. In Fig. 3b it can be seen that sample specimens S_4 and S_{19} are detected over the regulated limit of 0.1 ng/mL for benzo(b) fluoranthene In Fig. 3c we see that the two sample specimens really polluted with benzo(k)fluoranthene over the 0.1 ng/mL level have been detected by the SSC technique. Even the sample S_{14} which is very close to this limit has been detected. Also the sample specimen S_4 having high contamination by benzo(a)pyrene was accurately detected (Fig. 3d) as well as the S_{14} sample which is over the 0.1 ng/mL. Sample S_4 is detected as contaminated by benzo(g,h,i)perylene and Indeno(1,2,3-c,d)pyrene (Fig. 3e and f) and sample S_{22} was detected as contaminated by indeno(1,2,3-c,d)pyrene. However, sample S_{19} that was polluted by these two PAHs at 0.2 and 0.1 ng/mL levels respectively, were not detected.

In practical cases, if a sample specimen in a set of samples is predicted clearly over the regulated limits the sample is marked as contaminated no matter the component de-

tected because regulations define limits for the sum of PAHs. However, the goals and requirements of the sampling campaign and the environmental study will dictate later decisions relating these samples. Critical samples are those that, as the sample specimen S_{19} in our experiment, show evidences of contamination but the exact concentration levels are needed to decide how to classify that samples. In that case, usually, those samples should be analysed individually to gain confidence in the results and to take more accurate decisions.

It should be noticed that results for sample specimen S_8 are excellent in all cases although this sample specimen was spiked at very low concentration levels. The ability of SSC to detect these very small quantities of PAHs is related to the double proportion assigned to specimen S_8 thus showing the importance of proportional sample composition.

SSC in any case has provided important and accurate information with a limited analytical effort. In our experiment the number of analyses carried out was less than a half of the sample specimens available and only sample S_{19} remains with some doubts after the study, making advisable an additional determination.

4. Conclusions

In environmental screening studies, where minimum cost and analysis time for large series of sample specimens is critical, strategic sample composition can be a valuable technique that allows a significant reduction of the number of analyses to be carried out, thus producing direct savings in time, cost and manpower. This is especially true when sample preparation is labour intensive and analytical procedures are expensive and time consuming, which is the case of most environmentally concerning compounds and chromatographic procedures. If automation devices such the one presented in this paper are available all the process can be conducted unattended and free of errors. Depending of the detection limits allowable, more or less large design matrices can be applied, although 2:1–3:1 ratios between columns and rows in the composition matrix provide excellent performance. Samples having concentration levels around the regulated limits frequently need an individual confirmation of the predicted results to take accurate decisions.

Acknowledgements

The Spanish Science and Technology Ministry and the Local Government of Galicia have supported the development of SSC, under the projects REN2000-0984/HID and PGIDT01PXI23701PN, respectively. L. Pensado and E. Martinez acknowledge their doctoral grants to the Local Government of Galicia.

References

[1] European Community Directive 98/80/EC, 3-11-98, on the quality of water for human consumption, Official Journal European Communities no. L330/32 05/12/1998.
[2] Clean Water Act, codification 33 U.S.C. 1251-et seq., P.L. 95-217, 28 December 1977, Water Quality Act P.L. 100.4, 4 February 1987, amendments 1997 and National Drinking Water Standards, Office of Water, EPA 816-F-02-013, July 2002.
[3] Toxicological profile for polycyclic aromatic hydrocarbons (PAHs), Agency for Toxic Substances and Disease Registry (ATSDR), US Department of Health and Human Services, Public Health Service, Atlanta, GA, 1995.
[4] G.P Patil, Composite sampling, in: A H. El-Shaarawi, W.W. Piegorsch (Eds.), Encyclopaedia of Environmetrics, vol. 1, Wiley, Chichester, 2002, p. 387.
[5] EPA Observational Economy Series, vol. 1, Composite Sampling, USA Environmental Protection Agency, EPA-230-R-95-005, August 1995.
[6] R. Cela, E. Martinez, A.M. Carro, Chemom. Intell. Lab. Syst. 52 (2000) 167.
[7] R. Cela, E. Martinez, A.M. Carro, Chemom. Intell. Lab. Syst. 57 (2001) 75.
[8] E. Martinez, R. Cela, A.M. Carro, J.C. Cobas, B. Garcia, J. Anal. Atom. Spectrom. 17 (2002) 1373.
[9] E. Martinez, P. Landin, A.M. Carro, M. Llompart, R. Cela, J. Environ. Monit. 4 (2002) 490.
[10] E. Martinez, M. Pazos, A.M. Carro, M. Llompart, R. Cela, J. AOAC Int. 86 (2003) 846.
[11] J.B. Quintana, E. Martinez, A.M. Carro, R.A. Lorenzo, R. Cela, Int. J. Environ. Anal. Chem. 83 (2003) 269.
[12] R. Rodil, E. Martinez, A.M. Carro, R.A. Lorenzo, R. Cela, LCGC North America 22 (3) (2004) 272.
[13] D.K.J. Lin, Technometrics 37 (1995) 191.
[14] W.W. Lin, C.F.J. Wu, Technometrics 39 (1997) 171.
[15] G.A. Lewis, D. Mathieu, R. Phan-Tan-Luu, in: J. Swarbrick (Ed.), Pharmaceutical Experimental Design, Marcel Dekker, New York, 1999.
[16] J. Lintelmann, W.J. Günther, E. Rose, A. Kettrup, Fresenius J. Anal. Chem. 346 (1993) 988.
[17] L.H. Keith, Environmental Sampling and Analysis: A Practical Guide, Lewis Publishers, NY, 1991, Chapters 2 and 4.